of force alternans to peak force was at least tenfold larger than the ratio of AP phase II voltage alternans to maximum AP phase II voltage. In conclusion, electrical and mechanical alternans are both rate-dependent and linked via abnormal calcium handling, but mechanical alternans has the greatest amplitude across all pacing rates. Thus, mechanical alternans, due to their greater SNR, may be better predictors for arrhythmogenic propensity in heart failure patients than electrical alternans.

2543-Plat

Na^+/H^+ Exchange Blockers Reveal the Existence of a Skeletal Muscle Ca^{2+}/H^+ Exchanger, which is Altered in Malignant Hyperthermia Muscle Cells

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In resting skeletal muscle fibers intracellular pH (pHi) is kept constant at a relatively alkaline level. The transporters involved in maintaining muscle pHi at rest are the Na^+/H+ exchange system (NHE), and to a lesser extent the Na^+- and Cl–dependent bicarbonate dependent transport systems. Many studies conducted in nerve and smooth cells have suggested a link between changes in intracellular free Ca^{2+} ($[Ca^{2+}]i$) and changes in intracellular pH (pHi) suggesting a Ca^{2+}/H+ exchange (Daugirdas et al, 1995). Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle triggered by inhalational anesthetics and depolarizing neuromuscular blocking agents. We have measured $[Ca^{2+}]i$ and pHi simultaneously in Wt and heterozygous R163C myotubes using double barreled Ca^{2+}-selective and single barreled pH+-selective microelectrodes. MH cells have a higher $[Ca^{2+}]i$ and a lower pHi than Wt cells. Treatment of both MH and Wt cells with the NHE inhibitors dimethylamiloride (DMA) or cariporide, caused an increase in $[Ca^{2+}]i$ and a decrease in pHi in a dose dependent manner. These effects were more prominent in MH than Wt myotubes. YM-244769, a high affinity reverse mode NCX3 blocker, did not modify $[Ca^{2+}]i$ overload or the drop in pHi elicited by DMA or cariporide in either cell type, suggesting that the change in $[Ca^{2+}]i$ was not the consequence to an activation of the reverse form of the exchanger. Gd3+ and dantrolene respectively were able to partially inhibit or fully reverse the DMA or cariporide-mediated elevation of $[Ca^{2+}]i$ and acidification in both types of cells. These results suggest the existence of a Ca^{2+}/H+ exchange in skeletal myotubes, which appears to be altered in MH muscle cells.

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Calcium Sparklets in Intact Mammalian Skeletal Muscle Fibers Expressing the Embryonic CaV1.1 Splice Variant

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The embryonic splice variant of the voltage-gated L-type calcium channel (CaV1.1e) displays an altered voltage-dependence and gating kinetics as compared to that expressed in adult skeletal muscle. Because the adult CaV1.1a only opens slowly at strong depolarizations, its contribution as a source of calcium influx during action potentials is negligible. In contrast, calcium influx through the embryonic CaV1.1e substantially contributes to depolarization-induced calcium transients in fetal muscles and in cultured myotubes. In a genetically modified mouse (CaV1.1αE29), which exclusively expresses the embryonic CaV1.1e variant also in adult muscle the calcium influx component is maintained throughout life. Utilizing this mouse model, calcium release events - calcium sparklets - were recorded in enzymatically isolated, intact adult skeletal muscle fibers from the m. flexor digitorum longus using the fluorescent calcium probe fluo-8 and the fast confocal scanner (ZeissLive) in the x-y mode. While control animals did not display such events, CaV1.1αE29 mice spontaneously generated sparklets with a frequency of 9.2*10-4 ± 6*10-4 Hz/μm2 (19 fibers; mean ± SEM). The role of external calcium as the trigger was tested by either removing calcium from the external solution or by the application of 5μM nizoldipine to block the calcium current through CaV1.1e. Both interventions resulted in a complete loss of the events. Identified sparklets (n=311) were characterized by an average amplitude (αF/F0) of 0.287 ± 0.005, a full-width at half-maximum of 3.05 ± 0.05 μm, and duration of 235 ± 4 ms, clearly different from the properties of calcium sparks on saponin-permeabilized adult mammalian skeletal muscle fibers. These findings indicate that the sustained expression of the CaV1.1e splice variant gives rise to spontaneous calcium entry events (sparklets) in adult muscle fibers and that their properties are distinct from calcium sparks arising from ryanodine receptors. Support: OTKA-NN107765, FWF P23479, W1101, TÁMOP-4.2.4.A./2-11-1-2012-0001.

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Calcium Channel Dysfunction in a Mutant Mouse Model of Malignant Hyperthermia(CaV1.1 R174W)

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Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder of skeletal muscle that is triggered by exposure to volatile anesthetics. MH has been studied extensively in mice and pigs carrying causative mutations in the type 1 ryanodine receptor (RyR1). However, no in vivo information exists regarding how mutations in the skeletal muscle L-type Ca^{2+} channel (CaV1.1) precipitate MH crises. For this reason, we generated a mouse line carrying the R174W mutation. Homozygous R174W mice ambulated efficiently, reproduced and had normal lifespans. When exposed to isoflurane, homozygous R174W mice entered a hypermetabolic state ending ultimately in death. On the ultrastructural level, R174W muscle displayed limited and variable changes: some variability of the SR calsequestrin content, displacement of mitochondria in some soleus fibers of aged mice and occasional accumulation of SR stacks. On the cellular level, homozygous R174W muscle had elevated resting myoplasmic Ca^{2+} levels that were greatly increased upon exposure to isoflurane. Flexor digitorum brevis (FDB) fibers dissociated from homozygous R174W mice lacked L-type Ca^{2+} current even though intramembrane charge movements of were of similar magnitude and voltage-dependence to those recorded from wild-type fibers. Ca^{2+} released from the SR in response to depolarization was substantially reduced in homozygous R174W fibers suggesting a depleted SR Ca^{2+} store. Lipid bilayer recordings showed that the Po of RyR1s isolated from homozygous R174W mice was significantly increased at all cis Ca^{2+} concentrations (Feng et al., this meeting). Taken together, our results support a mechanism for MH susceptibility in which CaV1.1 R174W promotes SR Ca^{2+} leak without affecting EC coupling per se. This work was supported by grants from the NIH (AR055104 to KGB, AR052534 to PDA, KGB, PMH, CFA and INP) and MDA (MDA277475 to KGB). DB received a stipend from 2T32AG000279-11.

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Spatially Localized Disruptions of Voltage Activated Calcium Release in Mtm1-Deficient Muscle Fibers

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Mutations in the gene encoding the phosphoinositide phosphatase myotubularin (*Mtm1*) are responsible for myotubular myopathy. We previously showed that muscle fibers from *Mtm1*-deficient mouse suffer from defective excitation-contraction coupling. Here we measured voltage-clamp activated Ca^{2+} signals in fibers from the flexor digitorum brevis muscles of 4 week-old wild type (WT) and *Mtm1*-ko mice under line-scan confocal microscopy using the dye rhod-2. Ca^{2+} release in the diseased fibers was deficient over the full range of activation: fitting the voltage dependence of the peak rate of rise of the line-averaged rhod-2 F/F_0 signals with a Boltzmann function gave mean values for maximum rate, midpoint voltage and slope of 0.29 ± 0.02 and 0.14 ± 0.02 $F/F_0.ms^{-1}$, −8.8 ± 1.5 and 0.95 ± 2.3 mV, 7.0 ± 0.4 and 9.2 ± 0.7 mV in WT (n=22) and *Mtm1*-ko fibers (n=15), respectively. Furthermore, the mean time to peak rate was significantly delayed by 5-10 ms in *Mtm1*-ko as compared to WT fibers. These global alterations were associated with severe spatial inhomogeneity of Ca^{2+} release in the diseased fibers with rhod-2 transients yielding localized disruptions along the scanned line including reduced peak amplitude but also delayed or slower rate of onset, suggestive of specific alteration of the early peak component of the rate of Ca^{2+} release. In fibers treated with wortmannin and LY294002, the properties of the line-averaged rhod-2 F/F_0 signals were unchanged in WT fibers but the mean maximum rate of rise of the rhod-2 signal was enhanced by 60% in *Mtm1*-deficient fibers. Results show that *Mtm1*-deficiency provokes EC coupling failure through accumulation of spatially localized disruptions of